GUAM ENVIRONMENTAL PROTECTION AGENCY

STANDARD OPERATING PROCEDURE

Enterolert Enterococci Water Analysis

Signature and Title

Prepared by: _____ _____
Date

Approved by: _____ _____
Date

1.0 Scope and Application

Enterolert is used for the detection and quantification of enterococci in water. It may be used in drinking water, fresh and marine surface waters and wastewaters. There are no limits for enterococci in the National Primary Drinking Water Regulations for public water supplies or the Surface Water Treatment Rule promulgated under the Safe Drinking Water Act. There are no methods for enterococci analysis in National Pollutant Discharge Elimination System (NPDES) wastewater discharges promulgated under 40 CFR Part 136.

2.0 Method Summary

The Enterolert test is based upon the ability of enterococci to metabolize a media substrate producing a fluorescent substance. Quantification is performed using the IDEXX Quanti-tray Most Probable Number (MPN) system. According to the manufacturer, Enterolert can detect 1 enterococcus bacteria in a 100 mL sample.

3.0 Definitions

1. Enterococci - The enterococcus group is a subgroup of fecal streptococci that includes S. *faecalis, S. faecium, S. gallinaum and S. avium.* Enterococci are a valuable indicator for determining the extent of fecal contamination in recreational surface waters. Enterococci are differentiated from other streptococci by their ability to grow in *6.5%* sodium chloride, at ph 9.6, and at 10 °C and *45* °C.

2. Enterolert - Enterolert is a product of IDEXX laboratories, Inc. (800-321-0207).

3. MPN - Enterolert can be used as either a presence/absence test, or for enumeration of Most Probable Number (MPN) per 100 ml. Enumeration is possible using either multiple tubes, as in a traditional test, or using IDEXX's Quanti-trays.

4.0 Health and Safety Warnings

1. Microbiological analyses involve the culturing of potentially pathogenic organisms. Gloves, lab coats and safety glasses should be worn when handling samples, culturing media and equipment. All biologically contaminated materials in the laboratory, particularly media with growth, must be autoclaved prior to disposal. Contaminated media must never be discarded in the trash or dumped down the drain prior to autoclaving. Laboratory equipment and benches should be disinfected daily.

2. All laboratory-acquired infections must be reported to the Laboratory Director, as must

all accidents which may cause infection such as: accidental inoculation with syringes and needles; accidental oral aspiration of infectious material through a pipette; and spilling or spattering of pathogenic cultures on floors, table tops and other surfaces.

3. A 6-watt ultraviolet light is used to read Enterolert results. Care should be taken not to look directly at the light, and it should be pointed away from the analyst during readings.

5.0 Handling & Preservation

Samples for microbiological analysis should be collected using aseptic sampling procedures. Samples should be held at 4 °C during transit to the laboratory. Analysis must begin within 8 hours of sample collection.

6.0 Interferences

1. The manufacturer recommends diluting marine water samples at least 1:10 with sterile fresh water in order to reduce the possibility of interference by marine bacilli.

2. If chlorinated water is to be analyzed, sterile sample bottles must contain sodium thiosulfate to neutralize any residual.

7.0 Apparatus and Materials

Enterolert dry media in "Snap-Packs," stored in the dark at 4-30°C.
Quanti-Tray 51 well and/or Quanti-Tray 2000 MPN trays.
Quanti-Tray Sealer
Incubator at 41 ± 0.5°C
6 watt, *365* nm UV lamp
Quanti-tray MPN Tables

Sterile Disposable Pipettes
Sterile 90 or 99 mL Dilution Water Bottles

8.0 Quality Control Procedures and Limits

1. A sterile laboratory blank should be run with each days samples.

2. Positive and negative controls should be run on each new lot of Enterolert. The expected results for various types of bacteria are as follows:

Organism	Expected Result
Enterococcusfaecium, Streptococcus faecalis	fluorescent
Serratia marcescens, E. coli	non-fluorescent
Aerococcus viridans, Staphylococcus aueralis	non-fluorescent

9.0 Procedures

9.1 Dilution of Samples

1. Dilutions are recommended for marine samples and/or for enumerating samples with MPNs greater than 2400. Shake sample bottles vigorously 25 times before removing aliquots for preparing dilutions.

2. The manufacturer of Enterolert recommends diluting marine samples at least 1:10. To prepare a 1:10 dilution either use a 90 mL sterile dilution water bottle or remove and discard 9 mL from a prepared 99 ± 2 mL sterile dilution water bottle using a 10 mL pipette. Then using the same pipette add 10 mL of sample to the dilution water bottle.

3. Prepare 1:100 or 1:50 dilutions by adding 1 or 2 mL of sample to a sterile 99 mL dilution water bottle. Natural waters generally have enterococci MPNs below 2400. Wastewaters may have enterococci MPNs above 1 million.

9.2 Presence/Absence Test Procedure

1. Carefully separate one Enterolert Snap Pack from the strip taking care not to accidentally open adjacent pack.

2. Tap the Snap Pack to ensure all of the Enterolert powder is in the bottom part of the pack.

3. Open one pack being careful not to touch the opening of the pack.

4. Add the reagent to the water sample in a sterile, transparent, non-fluorescent 100 mL vessel.

5. Aseptically cap and seal the vessel.

6. Shake until dissolved.

7. Incubate for 24 hours at 41 ± 0.5 °C.

8. Read the results at 24 hours. Check the vessel for fluorescence by placing a 6 watt *365*

nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If fluorescence is observed the sample is positive for enterococci. If the endpoint is unclear, compare the result against Enterolert dispensed into an identical incubated vessel containing sterile water.

9.3 MPN Enumeration Test Procedure

Enterolert can be used for multiple tube Most Probable Number (MPN) analyses using serial dilutions as in the standard MPN test. However it is easier and more accurate to use the 51 well Quanti-Trays for MPNs from 0 to 200, or the Quanti-Tray 2000 for MPNs from 0 .2400. For counts above 2400/100 mL, the sample must be diluted as described in section 9.1 above. After the sample or dilution has been prepared, follow steps 1-6 above for the Presence/Absence test and then proceed as follows:

1. Pour the sample reagent mixture from step 6 into a Quanti-Tray or Quanti-Tray 2000 avoiding contact with the foil tab. Seal the tray according to the instructions on the Quanti-Tray sealer. Be sure to use the correct rubber insert in the sealer for either the Quanti-Tray or Quanti-Tray 2000.

2. Incubate for 24 hours at 41 ± *0.5* °C.

3. Follow the same interpretation directions from step 8 above to count the number of positive wells. The large well at the top of the Quanti-Tray is counted as one well. Refer to the Quanti-Tray and Quanti-Tray 2000 MPN Tables to determine the Most Probable Number of enterococci in the sample or dilution. The color and intensity of positive wells may vary.

Procedural Notes

1. If an inoculated Colilert sample is inadvertently incubated more than 28 hours, the following guidelines apply: Lack of color is a valid negative test. A yellow color after 28 hours is not valid and should be repeated or verified.

2. Some water samples containing humic material may have an innate color. If a water sample has some background color, compare the inoculated water Colilert sample to a control blank of the same water sample.

3 Colilert is already buffered and does not require the use of buffered water for dilutions. Always add Colilert to the proper volume of diluted samples after making dilutions.

10.0 Data Acquisition, Reduction, and Documentation

The results for presence/absence and quantification of total coliforms and E. Coli are determined according to the procedures above. A yellow color is confirmed positive for total coliforms, and both yellow and fluorescent is positive for E. Coli. Results are entered onto Guam EPA's Laboratory Colilert Data Sheets. Positive results are recorded with a plus sign (+)

and negative results with a minus sign (-). If Colilert quantitrays are used, any positive wells indicate presence and the sample is recorded as positive. The number of positive wells are then counted (first yellow wells for total coliforms, and then yellow and fluorescent wells for E. coli), and the results are converted to the MPN for total coliforms and E. coli using appropriate Colilert Quanti-tray matrices. The MPNs are then entered into the data sheet.

Laboratory data reports for Colilert results will include presence/absence for total coliforms and E. coli for each sample, as well as MPN if Quanti-trays were used. Results for all QC samples will also similarly reported.

11.0 References

American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992.

IDEXX, "Colilert for IDEXX" product instructions. Number 06-01701-03, undated

US Environmental Protection Agency, Microbial Methods for Monitoring the

Environment, *EPA-60018-78-0* 17, Dec 1978

US Environmental Protection Agency, National Primaiy Drinking Water Regulations, 40 CFR Part 141, "Analytical Methods for Regulated Drinking Water Contaminants," 12/5/94

US Environmental Protection Agency, Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition, EPA 81 5-B-97-OO 1, March 1997